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Original Article

EVALUATION OF *IN VIVO* WOUND HEALING ACTIVITY OF *SOLANUM VIRGINIANUM* ROOT EXTRACT ON EXCISION AND INCISION WOUND MODEL IN RATS

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ABSTRACT

Objective: Evaluation of the wound healing activity of *Solanum virginianum* root extracts using excision and incision wound models.

Methods: Moisture content, Ash value, Acid insoluble ash value, Water-soluble ash value, and different extractive values were studied as physicochemical parameters. Qualitative screening of each extract was carried out to determine different types of phytochemicals. In addition, a pharmacological study was conducted on groups of rats to find the effectiveness of root extracts (aqueous and alcoholic) on the wound healing process.

Results: Following observations were noted after the physicochemical test: Moisture content, 3.2%; Ash value, 5.77%; Acid insoluble ash, 1.17%; Watersoluble ash, 7.27%; Alcohol soluble extractive, 16.77%; Water-soluble extractive, 17.02%. After a qualitative chemical examination of alcohol and aqueous root extract, alkaloids, carbohydrates, phytosterols, saponins, phenolics, proteins, and flavonoids were detected. The excision wound model showed wound contraction for the alcohol extract group on the 12th day at 90.78% and the aqueous group at 85.23%. In the incision wound model, the alcohol extract-treated group showed significant wound breaking strength compared to the control and the aqueous extract-treated group.

Conclusion: This study on the root was carried out to find evidence of the wound healing activity of its extracts. Results indicate the effectiveness of the extracts in enhancing the wound healing process in rat models. The result of qualitative screening enhances the future scope for various pharmacological approaches.

Keywords: Solanum virginianum, wound healing, Aqueous extract, Alcohol extract

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INTRODUCTION

The synthetic drugs, which hold the capability for the treatment of certain critical and chronic diseases, was preferred over any alternative medicine due to its speedy recovery [1, 2]. However, as time passes, a surge was observed in herbal medicine and the shift was towards alternative medicines extracted from plants, with the potential to act against many diseases [3, 4]. According to the estimation of the World Health Organisation, around 80% of the world's population was dependent on plant-based drugs for primary health care. So, plants with medicinal properties were explored, and their underlying molecular mechanisms were studied for standardization [1-4].

Some of the plants documented for medicinal properties were Aglaia roxburghiana Baliospermum montanum Willd., Calotropis procera, Carmona retusa (Vahl.), Curcuma longa, Cocculus hirsutus L., Elephanto pusseaber L., Radix paeoniae, Jatropha curcas, Solanum xanthocarpum [5, 6].

Solanum virginianum, also known as yellow berried nightshade or Indian nightshade, was a prickly diffuse bright green perennial herb [7]. It belongs to the family of Solanaceae. This herb was available throughout the country in dry places as a weed on roadsides and wastelands. Some of the vernacular names of this plant used in different regions were Kantakari (Bengali, Sanskrit), Bhoringani (Gujarati), Bhauringani (Marathi), and Kandiari (Punjabi). Ancient Ayurveda describes this plant as pungent, bitter, digestive, and astringent. Activities such as anti-asthmatic, alternative hypoglycaemic, hepatoprotective, anti-inflammatory, anti-pyretic, and anti-spasmodic were present in Solanum virginianum [8]. It may be considered as a weed in many places, but its medicinal properties make this plant popular from a pharmaceutical point of view. The chemical constituents of Kantakari were glycoalkaloids and sterols. Different parts of the plant, including the whole plant, contain chemicals like diosgenin, sitosterol, solasodine, solamargine, solasonine, solanocarpidine, solanocarpine (Solanine-S),

tomatidienol, fatty acid, chlorogenic acid, caffeic acid, quercetin, apigenin, histamine, and acetylcholine [6, 8].

A humble effort was made in this study to conduct the physicochemical, phytochemical, and pharmacological analysis of the root extract of *Solanum virginianum*. This work aims to evaluate and pharmacologically validate the effectiveness of the root extract in facilitating the wound healing process.

MATERIALS AND METHODS

Identification and collection of plant material

The whole plant of *Solanum virginianum* was morphologically identified and collected from the region of Gwalior during February and March. Some plants were collected from the adjacent areas of the ITM University campus and wastelands near Alkapuri, Gwalior (M. P., India). Field data of the plant, such as height, flower colour, and condition, were recorded for investigation. The plant was authenticated by the botanist from ITM University Gwalior on 03/8/2017 (Voucher No.15). In addition, the pharmacognostic and physicochemical character was analyzed for experimental authentication of the root and its powder, collected from the whole plant of *Solanum virginianum*. Results of these studies were compared with standard material as reference.

Physicochemical test

Physicochemical analysis: The plant material of *Solanum virginianum* was shade dried at a temperature range of 20° to 30° C for about 2 w. The dried sample was then powdered in a grinding mill. The obtained powder was used for physicochemical analysis and extraction using solvents.

Determination of moisture content

5g powdered drug was weighed and kept in a porcelain dish. Further, it was dried in the oven at 100-105 °C. Later, the drug was

cooled and stored in a desiccator. The loss in weight was recorded as moisture content [9, 10].

Determination of ash value

Total ash

About 2 gm of powdered drug was weighed accurately into a tarred silica crucible and incinerated at 450 °C in a muffle furnace until free from carbon. The crucible was cooled and weighed. The percentage of total ash was measured considering the air-dried substance. The formula used for determination of total ash value was calculated [10].

$$Percentage(\%) = \frac{(Z - X)}{Y} \times 100$$

Where,

X = weight of empty dish

Y = weight of drug

Z = (weight of empty dish + weight of ash)

Acid insoluble and water-soluble ash

Ash was boiled with 25 ml of 2N HCl. Then it was filtered, and the residue in the paper was transferred to the silica crucible and washed with hot water. The residue was again incinerated at a temperature around 450 °C to make it free from carbon. The crucible was cooled, and the insoluble acid ash (in %) was calculated. For water-soluble ash, HCL was replaced with water. The difference in the weight of the ash and the insoluble matter represents the water-soluble ash [11].

Determination of extractive values

Alcohol soluble and water-soluble extractive value

5 g of the air-dried powdered drug was weighed and macerated with 100 ml of 90% alcohol in a closed flask for 24 h. The solution was frequently shaken during the first 6 h and later allowed to stand for 18 h. Rapid filtration was done to avoid solvent loss, and 25 ml of the filtrate was evaporated at 105 °C in a Petri dish. Then, the alcohol soluble extract (in %) was determined for the air-dried drug [10, 11]. The procedure was the same as mentioned above, except that distilled water was used instead of alcohol. The water-soluble extract (in %) was calculated for the air-dried drug [10, 11].

Preliminary phytochemical screening

Detection of alkaloids

1 mg of extract was dissolved in few drops of acetic acid followed by Mayer's reagent. White precipitate indicates the absence of alkaloids [12].

Detection of carbohydrates and glycosides

Two different test solutions were prepared for Molisch's test and Bontrager's test to detect carbohydrates and glycosides [12, 13].

Detection of phytosterols

20 mg of extract was dissolved in 1 ml of chloroform, 1 ml of acetic acid, and 1 ml of anhydride acetate. The solution is heated for 2-3 min, which results in the conversion of the pink colour to the green colour solution, thereby indicating the presence of sterols [10].

Detection of saponins

1 mg of extract was diluted in 7-8 ml of distilled water to conduct the foam test. Frothing persistence indicated the presence of saponins [14].

Detection of phenolic compounds and tannins

The test solution was prepared for the Ferric Chloride test to detect the presence of phenolic compounds and tannins. The same test solution was used for the gelatin test on both extracts to detect tannins [14].

Detection of proteins and free amino acids

Standard procedures were followed from Kokate 1994 to prepare test solutions to conduct the Biuret test and Ninhydrin test. Tekuri *et*

al. 2019 conducted tests to detect protein and free amino acids in extracts [10, 15].

Detection of flavonoids

An alkaline Reagent test was conducted on both extracts to detect the presence of flavonoids. Procedure for this experiment was mentioned in Kokate 1994. Ghildiyal *et al.* 2014 conducted similar experiments for pharmacognostical studies of *Solanum surattence* [10, 11].

Pharmacological studies

Animal

For this study, healthy adult male wistar rats weighing between 150-200g were procured from Defence Research Development Establishment (DRDE), Gwalior. The animals were housed at standard experimental conditions at a temperature (25 ± 1 °C) with a relative humidity of $50\pm55\%$ on 12 h light and dark cycles. They were fed standard rodent chow (Ashirvaad brand, Chandigarh, India) and water *ad libitum* [16].

Ethical approval

Experiments were performed as per the Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA) after the approval of the experimental protocol by the Institutional Animals Ethical Committee (IAEC) of the School of Pharmacy, ITM University Gwalior.(IAEC Reference no. ITM/SOP/AH/CPCSEA/0003).

Chemicals and drugs

Soframycin was procured from Aventis Pharma Ltd India. Ketamine Hydrochloride, ethanol (Fisher Scientific Pvt. Ltd, India), and all other chemicals were of analytical grade.

Acute toxicity study

Acute toxicity study of the root of *Solanum Virginianum* was carried out according to the Organisation for Economic Co-operation and Development (OECD) guideline (423). A single dose of 300 and 2000 mg/kg of both aqueous and alcohol extract of the root of *Solanum virginianum* (L.) was administered to separate groups of animals [16]. The animals were observed continuously for two hours for signs like sedation, piloerection, tremors, and paw licking. After 24 to 72 h, these animals were observed for any lethality or death.

Animal grouping

The animals were divided into four groups of 5 animals each [17].

Group 1: Control group (no treatment)

Group 2: Standard group [Framycetin sulphate (Soframycin, Aventwas)]

Group 3: Extracts group treated with 200 mg of *Solanum virginianum* alcohol extracts (10% w/v in saline)

Group 4: Extracts group treated with 200 mg of *Solanum virginianum* aqueous extracts (10% w/v in saline)

Other than the extracts, no extra medication was used for the test groups

Excision wound model

For wound healing studies, rats of comparable age and weight were used for each experiment. First, the rats were anesthetized with Ketamine Hydrochloride (50 mg/kg bodyweight (b. w), intraperitoneally (i. p)) to remove a portion of the skin at the back. Next, the fur of the animals at the vertebral region was removed using hair removal cream (Reckitt Benckiser, Inc., UK). Finally, a tenrupee coin was used to create an impression, and the entire thickness of the skin was excised from that demarcated area. The wound cre, measured around 500 mm² [17].

The areas of wounds were measured with the help of a tracing paper and millimeter graph paper on the day of creation (0^{th} day). This procedure was followed subsequently on the 4^{th} , 8^{th} , and 12^{th} days. Then, each drug

was applied topically every day from excision till observation of complete epithelisation [17, 18]. The epithelization period was the number of days required to completely heal without residual wounds and scars [19]. Observation regarding the healed area and percentage wound contraction was made on the 4th, 8th, and 12th days.

The Calculation for the wound Contraction area was given below [19]:

Wound Contraction(%) = $\frac{\text{Original area} - \text{final area}}{\text{final area}} \times 100$

Where the final area of the measurement of the wound was taken on a particular day

Incision wound model

In this model, the grouping of animals and the order in which they were treated were kept the same as in the excision wound model. The rats were anaesthetized with Ketamine Hydrochloride (50 mg/kg, i. p.), and furs were shaved as done in the excision wound model. After the initial procedure, a six cm paravertebral incision was made through the full thickness of the skin at a distance of 1 cm from the midline on either side of the vertebral column. The wound was adequately closed and tightly held together with uninterrupted surgical sutures. The stitching was done using black braided silk(no. 00) and a curved needle (no.11) at intervals of 1 cm. Treatment was applied to the wounds daily, topically twice a day for nine days, and at the 10th post-wounding day, the sutures were removed to measure the Wound Breaking Strength (WBS) [20, 21]. The breaking strength was measured using a continuous water flow technique where water was poured in a polyethylene bag to break the skin [22]. The rats were anaesthetized again on the 10th day, and lines were drawn 3 mm away from either side of the wound. Forceps facing each other were firmly applied on the line 3 mm away from the wound. One was fixed out of the two forceps, and the other was connected to a freely suspended lightweight polyethylene bag through a string run over to a pulley. The water was slowly and steadily poured into the container to increase its weight, resulting in a gradual increase in pressure to the wound site that ends in pulling apart its edges. The moment the wound opens up, no more water was poured into the bag, and the current weight was noted [20, 21].

Statistical analysis

Experiments were carried out in groups (n=5), and the results were reported as mean±standard error. The data was analyzed by one-way analysis of variance (ANOVA) and Tukey post-test. P-values<0.01 and P-values<0.001 were considered statistically significant.

RESULTS

Physicochemical test

The purity and the strength of the drug were determined through these tests, which creates an initial identity for selecting the drug. The results of moisture content, total ash, insoluble acid ash, watersoluble ash, alcohol soluble extractive, and water-soluble extractive were mentioned in table 1.

Preliminary phytochemical screening

This screening was done to determine the presence of different constituents in specific plant extracts. The chemical examination of each extract indicates the type of plant constituent present in it. Qualitative chemical examination of the root extracts detected the presence of alkaloids, phytosterols (steroids and tri-terpenoids), tannins, proteins, free amino acids, phenolic compounds, saponins, and flavonoids.

Table 1: Physicochemical characteristic of *Solanum virginianum* root

Parameters	Values (% w/w)
Moisture content	3.2
Total Ash	5.77
Acid insoluble ash	1.17
Water-soluble ash	7.27
Alcohol soluble extractive	16.77
Water-soluble extractive	17.02

 Table 2: Qualitative phytochemical screening of root extract of

 Solanum virginianum

Chemical constituent	Alcohol	Aqueous
Alkaloids		
Mayer's test	+	+
Carbohydrate and Glycosides		
Molwasch's Test	-	+
Bontrager's test	-	-
Phytosterols		
Liebermann-Burchard's test	+	-
Saponins		
Foam Test	-	+
Phenolic Compounds and Tannins		
Ferric Chloride Test	+	+
Gelatin Test	+	+
Proteins and free amino acids		
Biuret Test	+	+
Ninhydrin Test	+	+
Flavonoids		
Alkaline Reagent Test	+	+

'+' indicates presence, and '-'indicates absence.

Excision wound model

The alcohol extract-treated group was found more effective on the 4th, 8th, and 12th days than the aqueous extract-treated group. The healing percentage of the alcohol extract-treated group was statistically significant as compared to the control (P<0.001) and aqueous extract-treated group (P<0.01). The results for the soframycin treated group (Standard) was on expected lines, where a higher percentage of healing was observed as compared to the Control (P<0.001). In comparison with the aqueous extract-treated group, the results of the standard group showed significant statistical differences (P<0.01). Although the effect of the aqueous extract group was significantly lower than the standard group and the alcohol extract-treated group, it showed better results too as compared to the control(P<0.001). Both the extracts healed the wound on the 4th, 8th, and 12th day and had better results when compared to the control group. Efficacy of the alcohol extracttreated group was better than the two, where the healing percentage was closer to that of the standard group.

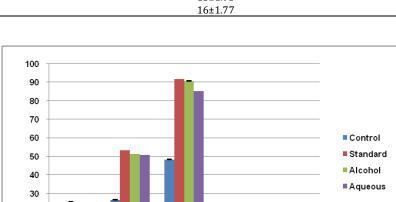
Table 3: Effect of root extracts of Solanum virginianum in wound contraction (in %) on excision wound model in rats

Group	4 th Day wound contraction (in %)	8 th Day wound contraction (in %)	12 th Day wound contraction (in %)
Control (Untreated)	13.99±0.313	26.59±0.096	48.37±0.133
Standard (Soframycin)	25.14±0.533 a***, d**	53.37±0.134 a***, d**	91.88±0.052 a***, d**
Alcohol extract (10% w/v)	24.93±0.220 a***	51.31±0.043 a***	90.78±0.073 a***,d**
Aqueous extract (10% w/v)	23.5±0.335 a***	50.85±0.077 a***	85.23±0.049 a***

All values are expressed in terms of mean±SEM (n=5). For statistical significance, groups are marked as a, b and c, where a: significant difference compared to group I, b: significant difference compared to group II, c: significant difference compared to group III, d: significant difference compared to group IV. **indicates P<0.01, **indicates P<0.001; considered as statistically significant.

Table 4: No. of days required for epithelisation of different groups

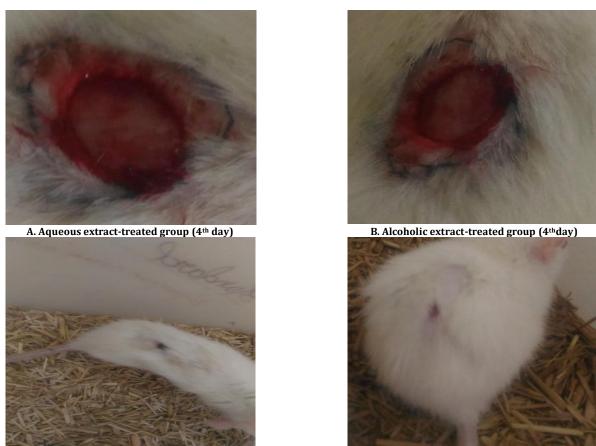
Groups	Period of epithelisation (in days)	
Control(untreated)	22±1.23	
Standard(Soframycin)	14±1.61	
Alcoholic Extract (10% w/v)	15±1.93	
Aqueous Extract (10% w/v)	16±1.77	



Graph 1: Excision Wound healing model depicts the rate of wound contraction (in %) and epithelisation (n=5). All values are expressed in terms of mean±SE

Epithelisation

12th Day



C. Aqueous extract-treated group (12th day)

20 10 0

4th Day

8th Day

D. Alcoholic extract-treated group (12th day)

Fig. 1: Ongoing wound healing process on 4th day and 12th day of Excision Wound model. Both aqueous and alcohol extract group contains five rats (n=5). A: Indicates the effect of the aqueous-extract treated group on the 4th day. B: Indicates the effect of the alcohol extracttreated group on the 4th day. C: Indicates the effect of the aqueous extract-treated group on the 12th day. D: Indicates the effect of the aqueous extract-treated group on the 12th day

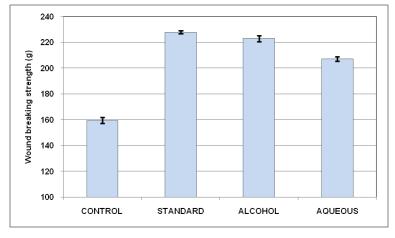
Incision wound model

The continuous water pouring technique was used to assess the tensile strength of the healed wound. Results suggest that wound breaking strength (in g) of the soframycin treated standard group was more. In contrast, the alcohol extract group showed significant breaking strength around the healed area. Moreover, the breaking strength of the alcohol extract treated group was found significantly higher than that of the aqueous extract-treated group. The alcohol extract-treated group showed significant concentration-dependent action in increasing the breaking strength than Control (P<0.01) and the aqueous extract-treated group (P<0.01). The aqueous extract-treated group as the breaking strength than the alcohol extract-treated group and standard (P<0.01), and significantly more as compared to the control (P<0.001).

Table 5: Effect of root extracts of *Solanum virginianum* in wound contraction (in %) on incision wound model in rats

Groups	Wound breaking strength (WBS)
Group-I: Control	159.50±2.26
Group-II: Standard	227.75±1.15 a***, **d
Group-III: Alcoholic extract(10% w/v)	222.90±2.19 a***, **d
Group-IV: Aqueous extract(10% w/v)	207.20±1.77 a***

The values for each group were represented as mean \pm SE. Each group consists of 5 rats (n=5). For statistical significance, groups were marked as a and d, where a: significant difference compared to group IV. **indicates P<0.01, ***indicates P<0.001; considered as statistically significant.



Graph 2: This graph depicts the level of the wound breaking strength (in g) shown by each group after removal of sutures on the 10th post wounding day. All values are expressed in terms of mean±SE. (n=5)



A: Alcoholic extract-treated group

B: Aqueous extract-treated group

Fig. 2: Incision wound model for alcoholic extract-treated and aqueous extract-treated groups. A: Alcoholic extract-treated group on 1st day (n=5) B: Aqueous extract-treated group on 1st day (n=5)

DISCUSSION

The purpose behind conducting physicochemical tests was to identify and standardize the purity of the drug. Various parameters like moisture content, acid insoluble ash, extractive values, etc., were studied under these tests to determine the purity of the drug. Ayurvedic Pharmacopoeia of India, Volume 1 suggests total ash value not more than 9%, Acid-insoluble ash value not more than 3%, Alcoholic-soluble extractive value not less than 6% and, Watersoluble extractive value not less than 6% and, Water soluble extractive value not less than 6% [23]. Analysis of the data on *Solanum virginianum* in the Ayurvedic Pharmacopoeia of India presents ample proof that the results of this experiment were in line with the minimum or maximum range mentioned in the booklet. Meena *et al.* 2010 corroborated the findings of the

physicochemical characteristics of *Solanum xanthocarpum*. The total ash value was 7.77%, acid-insoluble ash was 1.15%, water-soluble extractive was 21.22%, and alcohol soluble extractive was 22.28%; all values were in w/w [12]. The findings were parallel with the observations made in the previously mentioned work. The difference between the results was due to the environmental factors and geographical location of the whole plant.

Ghildiyal *et al.* 2014 mentioned the phytochemical screening of aqueous and ethanolic extracts of *Solanum surattence*, which states alkaloids, carbohydrates, tannins, flavonoids, starch, and glycosides were present [11]. Meena *et al.* 2010 reported phytochemical screening on different extracts of the same plant and found the presence of alkaloids, coumarin, steroid, flavons tannin, glycoside,

and saponin [12]. Gangwar *et al.* 2013 deduced the presence of flavonoids, saponins, proteins, and amino acids in aqueous extract but, these compounds were absent in the petroleum ether extract [14]. Husanappa *et al.* 2014 observed the presence of carbohydrates, tannins, alkaloids, steroids, proteins, and flavonoids in aqueous extract and the absence of the steroid in the alcoholic extracts [13]. This study suggests that qualitative chemical examination of the root extracts detected the presence of alkaloids, phytosterols (steroids and tri-terpenoids), tannins, proteins, and free amino acids, phenolic compounds, saponins, and flavonoids. The result of the current experiment indicates differences in comparison to the findings of the previously mentioned work due to the geographical location of the whole plant.

Dewangan et al. 2012 experimented to find the potential wound healing activity of ethanolic extract of Solanum xanthocarpum leaves. The study revealed that topical application of the ethanolic extract reduces the epithelisation period from 25 d to 19 d and the scar area from 53.88 mm^2 to 37.76 mm^2 . They stated that at a dose of 10% w/v in saline, the ethanolic extracts of the leaves show significant wound healing activity in the excision wound model [24]., Kumar et al. 2010 verified the effects of its fruits on experimentally induced excision wound models and the wound healing activity of Solanum xanthocarpum fruits. The percentage of wound healing in the methanolic extract-treated group, formulated at 10%w/w in white petroleum jelly, showed significantly better results than the control group [25]. Comparison and analysis of our research work with the previously mentioned authors observe that the alcohol extracttreated group consistently revealed better efficacy than the other extract-treated groups.

The findings of the excision wound model strengthen the observation made in the qualitative analysis of root extract. These extracts of Solanum virginianum showed the presence of alkaloids, flavonoids, carbohydrates, phenolics, phytosterols, and tannins. These secondary metabolites promote wound healing by multiple mechanisms, for example, wound contraction, increased rate of epithelialization, and prevention of secondary bacterial infection [26]. Both flavonoids and phenolics were important for astringent, anti-inflammatory, and anti-microbial activity [27]. These properties make them responsible for wound contraction and elevated rate of epithelisation. The extract's wound healing potency can be attributed to its phenolics and flavonoid content in this study. Some studies suggest that flavonoids and their derivatives were known to decrease lipid peroxidation by slowing down the process of necrosis [27]. There were reports which suggest that pro-anthocyanins and tannins were also capable of inducing wound healing [28]. In the case of tannins, the wound healing process was promoted through scavenging of free radicals, reactive oxygen species, formation of vessels, and fibroblasts [29, 30]. Presence of capillary phytoconstituents in different phases of the wound healing process can be the reason behind the effectiveness of root extracts of Solanum virginianum in wound healing.

In the incision wound model, the effect of alcoholic extract was noticeable, where the breaking strength on 10^{th} post wounding was significantly higher than the control and aqueous extract-treated group. Both extract-treated groups showed better efficacy in increasing the breaking strength than the control group, but the alcohol extract-treated group showed better results. Dewangan et al. 2012 suggest ethanolic of that extract the Solanum xanthocarpum leaves significantly increased the breaking strength in the incision wound model [24]. Kumar et al. 2010 reported the wound healing activity of Solanum xanthocarpum fruits in which the breaking strength in the methanolic extract treated group was significantly higher than the control group(376.50g>235.25g). The methanolic extract of Solanum xanthocarpum fruits possesses significant wound healing activity [25]. Some clinical studies suggest the critical role of collagen in wound repair. Putative action of collagen in wound repair are described with particular emphasis on haemostatic effect, interaction with platelets and fibronectin, properties of increasing fluid exudate and its cellular component (macrophages) and the "scaffold" role for fibroblastic proliferation. Collagen was responsible for increasing the tensile strength of the healing wound. It is secreted in the extracellular space in the form of pro-collagen, and, later, its

terminal segments were cleaved, which transforms it into tropocollagen [31, 32]. Tropocollagen makes collagen filaments by binding with other tropocollagen molecules. The filaments were rich in hydroxylysine and hydroxyproline content. It enables them to form strong inter and intra-molecular cross-linkages with each other. These links to other molecules, and proteins, hence increases the tensile strength of the wound. These fibres get deposited in a framework made up of fibronectin, which also acts as an anchor for myofibroblasts. These myofibroblasts later migrate to the wound site and play a part in wound contraction [31, 33].

CONCLUSION

This study mainly focussed on two extract groups, alcohol and aqueous, which after comparison with the standard and the control group, were found to be statistically significant. Results from the experiment using these extracts gave us evidence about the wound healing potential of the root extracts of *Solanum virginianum*. The research work provides pharmacological validation to the claims made in the folklore about the root of this plant.

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Nil

AUTHORS CONTRIBUTIONS

Mr. Anurag Agrawal: Associate Professor, School of Pharmacy. Contributed in the design of the experiment, guided in animal handling for conduction of experiment on rat model and preparation of manuscript.

Padmanava Chakraborti: M. Tech, Biotech Student, performed bench work, animal experiments and statistical analysis.

Dr. Sonia Johri: Associate Professor, School of Sciences guided in experimental design, biochemical analysis and preparation of manuscript.

CONFLICT OF INTERESTS

There was no conflict of interest in this work.

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